Mutation in *IRF2BP2* is responsible for a familial form of common variable immunodeficiency disorder

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Background: Genome-wide association studies have shown a pattern of rare copy number variations and single nucleotide polymorphisms in patients with common variable immunodeficiency disorder (CVID), which was recognizable by a support vector machine (SVM) algorithm. However, rare monogenic causes of CVID might lack such a genetic fingerprint.
Objective: We sought to identify a unique monogenic cause of familial immunodeficiency and evaluate the use of SVM to identify patients with possible monogenic disorders.
Methods: A family with multiple members with a diagnosis of CVID was screened by using whole-exome sequencing. The proband and other subjects with mutations associated with CVID-like phenotypes were screened through the SVM algorithm from our recent CVID genome-wide association study. RT-PCR, protein immunoblots, and *in vitro* plasmablast differentiation assays were performed on patient and control EBV lymphoblastoids cell lines.
Results: Exome sequencing identified a novel heterozygous mutation in *IRF2BP2* (c.1652G>A:p.[S551N]) in affected family members. Transduction of the mutant gene into control human B cells decreased production of plasmablasts *in vitro*, and *IRF2BP2* transcripts and protein expression were increased in proband versus control EBV-immortalized lymphoblastoid cell lines. The SVM algorithm categorized the proband and subjects with other immunodeficiency-associated gene variants in *TACI*, *BAFFR*, *ICOS*, *CD21*, *LRBA*, and *CD27* as genetically dissimilar from polygenic CVID.
Conclusion: A novel *IRFBP2* mutation was identified in a family with autosomal dominant CVID. Transduction experiments suggest that the mutant protein has an effect on B-cell differentiation and is likely a monogenic cause of the family's CVID phenotype. Successful grouping by the SVM algorithm suggests that our family and other subjects with rare immunodeficiency disorders cluster separately and lack the genetic pattern present in polygenic CVID cases. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Common variable immunodeficiency, machine learning, primary antibody deficiency,* IRF2BP2*, immunoglobulin*

Common variable immunodeficiency disorder (CVID) is one of the most frequently diagnosed forms of primary immunodeficiency that requires clinical intervention.[1-3] Defined clinically by a low quantity of 2 immunoglobulin classes (including IgG) and poor specific antibody production, it has been thought of as an umbrella diagnosis because of the heterogeneity in onset and comorbidities, including autoimmune disease and risk of malignancy.[4] Roughly 5% of cases are familial, and the increasing availability of next-generation sequencing technology has permitted identification of an increasing number of causative and associated mutations in patients with CVID-like disease.[5-16]

A recent multi-institutional genome-wide array study of CVID showed unique associations with specific single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), with intraexonic duplications in *ORC4L* being most highly associated with disease.[17] The studied CVID cohort was found to have a unique pattern of SNPs and rare CNVs, and a support vector machine (SVM) algorithm was successfully used to identify this pattern in patients with CVID versus control subjects. SVM is a learning algorithm used for nonlinear classification and regression analysis.[18] It can be trained with a variety of data and produces a "hyperplane" for subsequent classification. In the original study the CVID SVM hyperplane successfully classified cases with an accuracy of 91%, positive predictive value of 100%, and negative predictive value of 96%.

Although the SVM results support the polygenic nature of CVID, it was unclear whether patients with monogenic causes of CVID-like disease or risk alleles for CVID (*TACI* and *BAFFR*) would lack the genetic fingerprint of the more common polygenic disease. If this were the case, SVM classification of microarray

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Supported by an Institute Development Fund from CHOP (U01HG006830), a donation to CAG from the Kubert Estate Foundation, and the Jeffrey Modell Foundation.

Disclosure of potential conflict of interest: I. K. Chinn receives research funding from the National Institutes of Health and the Jeffrey Modell Foundation. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 17, 2015; revised January 1, 2016; accepted for publication January 13, 2016.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.01.018

Abbreviations used
CNV: Copy number variation
CVID: Common variable immunodeficiency disorder
EBV-LCL: EBV-immortalized lymphoblastoid cell line
SNP: Single nucleotide polymorphism
SVM: Support vector machine

data could be used to separate monogenic disorders from the more common polygenic form of CVID and could therefore be a useful screening method for identifying cases in which next-generation sequencing would be high yield, thereby guiding the proper workup for unknown CVID cases.

METHODS

Human sample collection, DNA extraction, and EBV-immortalized lymphoblastoid cell line production

A family with 3 affected patients with CVID and unaffected family members were enrolled in a research protocol approved by the host institutional review board (Children's Hospital of Philadelphia). DNA samples from patients with monogenic immunodeficiency disorders with CVID-like phenotypes were provided by collaborating researchers. DNA was extracted from peripheral blood with a DNeasy extraction kit (Qiagen, Hilden, Germany). EBV-immortalized lymphoblastoid cell lines (EBV-LCLs) were generated from PBMCs of 1 subject carrying the *IRF2BP2* mutation and 2 control subjects without the mutation by using a previously published protocol.[19]

Genetic studies

Whole-exome sequencing with the Agilent SureSelect Human All Exon 50Mb Kit (Agilent Technologies, Santa Clara, Calif) was performed on the proband and her family. Variants were matched to disease segregation (which suggested a heterozygous autosomal dominant pattern), and further narrowed by means of exclusion of synonymous mutations, *in silico* analysis of mutation effect, tissue expression pattern (BioGPS.org), and ties to known immunologic pathways. Variants with a minor allele frequency of greater than 0.5% in public databases (1000 Genomes and the National Heart, Lung, and Blood Institute's 6500 exomes Project) were excluded, as were variants that were previously identified in control subjects in our in-house exome variant database.

High-throughput SNP genotyping was performed with the Infinium HumanHap610 BeadChip (Illumina, San Diego, Calif), and the PennCNV algorithm was used for CNV calls. An SVM algorithm was trained with data from 179 patients with CVID and 1917 control subjects by using the 658 most significantly associated variants from the 2011 study.[17] Cytogenetic data from the proband and monogenic cases were subsequently analyzed by using the trained algorithm.

RT-PCR and immunoblotting

RNA was isolated from whole blood obtained from the patient and control subjects by using TRIzol (Applied Biosystems, Grand Island, NY) and RNeasy Kits (Qiagen). cDNA was produced with a high-capacity reverse transcriptase kit (Applied Biosystems, Foster City, Calif), and custom cDNA primers for *IRF2BP2* (both total and isoform 2) and *GAPDH* were created. Custom primers are detailed in Fig E1 in this article's Online Repository at www.jacionline.org. RT-PCR was performed with SYBR Green core reagents on a QPCR-7900HT system (Applied Biosystems). Gene dose was calculated by using the ΔΔ cycle threshold method.

For Western blot analysis of IRF2BP2 expression, EBV-LCLs were lysed with Nonidet P-40 lysis buffer (Invitrogen, Carlsbad, Calif). Proteins were separated on 4-12% NuPAGE Bis-Tris gels in MOPS SDS running buffer and transferred overnight onto nitrocellulose membranes (Invitrogen). The membrane was blocked in 3% BSA and cut into 2 halves. The top half was incubated with rabbit anti-IRF2BP2 polyclonal antibody (Abcam, Cambridge, United Kingdom), and the bottom half was incubated with rabbit anti-TATA-binding protein mAb (Abcam). Subsequently, the membranes were washed, incubated with secondary antibody for 1 hour, and washed again; bound antibody was detected with a WesternBright ECL chemiluminescence detection system (Advansta, Menlo Park, Calif).

In vitro plasmablast differentiation

Plasmablasts were produced from frozen PBMCs from the proband and healthy control subjects, as previously described.[20] Briefly, PBMCs were plated at 2.5×10^5 cells/well and treated with either CD40 ligand (Axxora, Farmingdale, NY) at 500 ng/mL, IL-21 (PeproTech, Rocky Hill, NJ) at 500 ng/mL, or CpG-ODN (InvivoGen, San Diego, Calif) at 2.5 μg/mL. Cells were harvested after 7 days of incubation and analyzed by means of flow cytometry with a FACSCalibur (BD Biosciences, San Jose, Calif). Cells were stained with CD19–fluorescein isothiocyanate, CD27–allophycocyanin, and CD38-phycoerythrin (BD Biosciences, San Jose, Calif). Isotype controls were also used. Results were analyzed with FlowJo software (TreeStar, Ashland, Ore). Plasmablasts were identified as $CD19^+CD27^+CD38^{++}$ cells with lymphocyte gating (see Fig E2 in this article's Online Repository at www.jacionline.org).

Constructs

Plasmids containing wild-type and mutant (p.S551N) *IRF2BP2* with a C-terminal Myc-tag were purchased (OriGene, Rockville, Md). Empty pCMV6 plasmid was also obtained for control purposes.

Isolation and transduction of human B cells

Primary human B cells were obtained from sorted PBMCs from healthy donors. Purity was confirmed by using flow cytometry. Cells were transduced by means of nucleofection with Nucleofector Program U-15 Amaxa and the Human B cell Nucleofector kit (Lonza, Allendale, NJ). Transduction was confirmed by using Western blotting for the Myc tag.

RESULTS

Clinical history

The proband is a 24-year-old woman who experienced recurrent sinopulmonary infections beginning in early childhood, which worsened in adolescence. She was evaluated immunologically and found to have gradually worsening hypogammaglobulinemia and poor vaccine responses between 17 and 19 years of age (Table I) and was given a diagnosis of CVID. She has been maintained on subcutaneous immunoglobulin with good infection control. She was given a diagnosis of CVID-related colitis at age 23 years after onset of chronic diarrhea and is being treated with Entocort (AstraZeneca, Wilmington, Del).

Her family history is notable for CVID in both her father and older brother (Fig 1). The proband's older brother was given a diagnosis of CVID at age 16 years after experiencing chronic sinus infections and has been treated with intravenous immunoglobulin therapy. He also has a history of type I diabetes. The proband's father was similarly given a diagnosis of CVID at age 16 years in the setting of recurrent sinus infections, which have been well controlled on intravenous immunoglobulin therapy. He also has a history of psoriasis. The paternal grandparents, aunts, and their families are unaffected, as is the maternal family.